



## Phytochemical Analysis of Traditional Medicine Herbs from Betel Leaf (*Piper betle*)L. to Treating Acne

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### ABSTRACT

Betel leaf (*Piper betle*L.) has been widely used for traditional medicine from generation to generation by the community, one of which is useful for treating acne. Betel leaf extract has antibacterial activity so that it can inhibit acne-causing bacteria. This study aims to determine the phytochemical compounds qualitatively and quantitatively. The herbs is made by boiling 8 betel leaves in 400 mL of water until it boils. The results showed that traditional medicinal herbs from betel leaf contain chemical compounds of flavonoids, tannins and phenols. Determination of the levels of phytochemical compounds using UV-Vis Spectrophotometry showed that the total flavonoid content was  $35.941\% \pm 0.997$ , total tannin was  $5.444\% \pm 1.357$ , and total phenol was  $2.905\% \pm 0.295$ . Flavonoid compounds are known to have antibacterial activity so they are thought to play a role in treating acne

Keywords: Betel leaf (*Piper betle* L.), Phytochemical, Acne

### INTRODUCTION

Traditional medicines are ingredients or ingredients in the form of plant ingredients, animal ingredients, mineral ingredients, extract preparations (galenic), or mixtures of these ingredients which have been used for generations for treatment and can be applied according to the norms that apply in society (Ministry of Health of the Republic of Indonesia, 2017). According to the Minister of Health of the Republic of Indonesia (2017) traditional medicinal herbs from betel leaves are used to treat acne, namely by boiling fresh betel leaves as much as 7-10 leaves with 2 cups of water until boiling, then the medicinal herbs are cooled, after cold the medicinal herbs are used for washing face 2 times a day. According to Dalimartha (2006) medicinal herbs from betel leaves are used to treat acne by mashing fresh betel leaves as much as 7-10 pieces until smooth. Then brewed with two glasses of hot water. Then cover and leave to cool. Then it is filtered and the results are used to wash the face with acne 2-3 times a day.

Empirically, betel leaf has been used extensively in the treatment of asthma and sore throat. Betel leaves are processed as herbal betel and drunk is believed to eliminate bad body odor, eliminate bad breath and medicine for nosebleeds. Betel leaf is widely used in the treatment of toothache and swollen gums. Betel leaves are also used in curing skin diseases such as dry eczema and wet eczema, and prevent and get rid of acne (Hakim, 2015).

Betel leaves contain 0.8-1.8% essential oil (consisting of chavikol, chavibetol (betel penol), allylpyrocatechol (hydroxychavikol), allylpyrocatechol-mono and -diacetate, carvacrol, eugenol, eugenol methylether, p-cymene, cineole, caryophyllene, candinene, estragol), terpenenes, sesquiterpenes, phenyl propane, tannins, diastase, carotenes, thiamine, riboflavin, nicotinic acid, vitamin C, sugars, starches, and amino acids. Chavikol, which causes betel nut, has a distinctive odor and has antibacterial properties (killing bacteria five times stronger than ordinary phenol) and immunomodulators (Dalimartha, 2006).

Green betel leaf extract (*Piper betle* L.) contains phenolic compounds and their derivatives which can inhibit the growth of *Propionibacterium* acnes. The antibacterial mechanism of phenolic compounds in killing microorganisms is by denaturing bacterial cell proteins (Carolia&Noventi, 2016). In a study conducted by Kursia et al., (2016) a betel leaf extract was tested using ethyl acetate solvent and its inhibitory activity was tested against *Staphylococcus epidermidis* using the agar diffusion method. The results of this study indicate that betel leaf extract using ethyl acetate solvent has antibacterial activity against *Staphylococcus epidermidis* in the medium-strong category.

Based on the description above, it turns out that a phytochemical analysis of traditional medicinal herbs from betel leaf (*Piper betle* L.) as a medicine to treat acne has never been carried out. Therefore, researchers are interested in conducting research on the phytochemical analysis of traditional medicinal ingredients from betel leaves (*Piper betle* L.) according to the Minister of Health of the Republic of Indonesia (2017).

## METHOD

### Tools

The tools used were a double beam UV-VIS spectrophotometer (Shimadzu UV-1800), analytical balance (Presica), oven (Mamert), UV lamp (Camag), filter paper, aluminum foil, evaporating cup, funnel (Pyrex), stove (Hock), spatula, suction ball, measuring pipette (Iwaki), test tube (Iwaki), drip plate, dropper pipette, bunsen, gauze, tripod, asbestos, laboratory glassware (Pyrex).

### Materials

The materials used were fresh betel leaves (*Piper betle* L.), Mg powder (Merck), Zn powder (Merck), Iron (III) chloride (FeCl<sub>3</sub>) (Merck), anhydrous acetic acid (CH<sub>3</sub>CO)<sub>2</sub>O (Merck), sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) (Merck), hydrochloric acid (HCl) (Merck), methanol Pa (CH<sub>3</sub>OH) (Merck), 96% ethanol (C<sub>2</sub>H<sub>5</sub>OH) (PT. Novalindo), aquadest (PT Novalindo), Aluminum chloride (AlCl<sub>3</sub>) (Merck), Sodium hydroxide (NaOH) (Merck), Potassium iodide P (KI) (Merck), Sodium acetate (C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub>) (Merck), n-Hexane (C<sub>6</sub>H<sub>14</sub>) (Merck), Vanillin P (C<sub>8</sub>H<sub>8</sub>O<sub>3</sub>) (Merck), FolinCiocalteu LP (C<sub>10</sub>H<sub>5</sub>NaO<sub>5</sub>S) (Merck), Rutin (C<sub>27</sub>H<sub>30</sub>O<sub>16</sub>), catechins (C<sub>15</sub>H<sub>14</sub>O<sub>6</sub>) (Sigma aldrich), and gallic acid (C<sub>7</sub>H<sub>6</sub>O<sub>5</sub>) (Merck).

### Sampling

The sample used in the study was betel leaf (*Piper betle*L.) obtained from Surau Gadang Village, Nanggalo District, Padang City, West Sumatra.

### Identification of Samples

Identification of plants was carried out at the Herbarium of Andalas University (ANDA), Department of Biology, Faculty of Mathematics and Natural Sciences (FMIPA) Andalas University (UNAND) Padang, West Sumatra. The samples identified were all parts of the plant.

### Preparation of Betel Leaf Herbs

Take 8 fresh betel leaves, then wash them thoroughly using running water, then boil the betel leaves with 2 cups of water equivalent to 400 mL of water over medium heat for 15 minutes in a clay container to boil, then cooled, after cold then filtered. So that we get the results of traditional medicinal herbs from betel leaves. And qualitative and quantitative tests were carried out on the medicinal ingredients (Ministry of Health of the Republic of Indonesia, 2017).

### Qualitative Analysis of Betel Leaf Medicinal Herbs

#### a. Alkaloid Test

A total of 5 mL of traditional medicinal herbs from betel leaves (*Piper betle*L.) was added to 1 mL of 2 N hydrochloric acid and 9 mL of water, heated over a water bath for 2 minutes, cooled and filtered. Pipette 3 drops of the filtrate to a test tube, add 2 drops of Bouchardat LP to form a brown to black precipitate, then there is a possibility that alkaloids are present. If with Mayer LP white or yellow precipitate, precipitate is formed which dissolves with methanol P. If with Wagner LP a reddish brown, precipitate is formed and with Dragendorf a reddish brown precipitate is formed (Department of Health of the Republic of Indonesia, 1995).

#### b. Flavonoids

- 1) Evaporate until dry 5 mL of traditional medicinal herbs from betel leaves (*Piper betle*L.), the remainder is dissolved in 1 mL to 2 mL ethanol (96%) P, add 0.5 g zinc powder P and 2 mL hydrochloric acid 2 N, let stand for 1 minute. Add 10 drops of concentrated hydrochloric acid, if within 2 to 5 minutes an intense red color occurs, indicating the presence of flavonoids (glycoside-3-flovanol).
- 2) Evaporate until dry 5 mL of traditional medicinal herbs from betel leaves (*Piper betle*L.), the remainder is dissolved in 1 mL of ethanol (96%) P, add 0.1 g of magnesium P powder and 10 drops of concentrated hydrochloric acid, if a red color occurs orange to purple red, indicating the presence of flavonoids. If an orange yellow color occurs, it indicates the presence of flavone, chalcone and auron (Ministry of Health of the Republic of Indonesia, 1995).

#### c. Tannin Test

Herbs *Piper betle* into 2 mL of distilled water. Then the solution was added with two drops of 1% FeCl<sub>3</sub> solution. The presence of tannins is indicated by the appearance of a dark green or bluish green color (Endarini, 2016).

#### d. Test of Terpenoids and Steroids

As much as 2 mL of traditional medicinal herbs from betel leaf (*Piper betle*L.) added 10 mL of n-hexane and then filtered. The solution obtained was taken 5 drops and dried on a drip spot board, added three drops of acetic anhydride and then one drop of concentrated sulfuric acid. The presence of terpenoid group compounds will be indicated by the appearance of a red color while the presence of steroid class compounds is indicated by the appearance of a blue color (Endarini, 2016).

**e. Saponin Test**

As much as 1 mL of traditional medicinal herbs from betel leaves (*Piper betleL.*) is added to 10 mL of water and shaken vigorously for 10 minutes, foam formed for not less than 10 minutes, 1 cm to 10 cm high. On the addition of 1 drop of 2 N hydrochloric acid, the foam did not disappear (Ministry of Health of the Republic of Indonesia, 1995).

**f. Phenol Test**

As much as 2 mL of traditional medicinal herbs from betel leaves (*Piper betleL.*), added with a concentrated Folin-Ciocalteu reagent solution will form a green-black or bluish color (Hanani, 2015).

**g. Essential oils**

- 1) Put 2 mL of the traditional medicinal herbs from betel leaf (*Piper betleL.*) into a test tube then add 2 drops of potassium permanganate solution, the color will turn pale or disappear.
- 2) Put 2 mL of the traditional medicinal herbs from betel leaf (*Piper betleL.*) into a test tube then add 2 drops of acetic acid anhydride, then carefully add 1 mL of concentrated sulfuric acid so that a blue-green color appears (Hanani, 2015).

**Quantitative Analysis of Betel Leaf Medicinal Herbs****a. Total Flavonoids**

As much as 10 mg rutin in 96% ethanol was put into a 10 mL volumetric flask, so that a concentration of 1000 µg/mL (standard solution) was obtained. Perform dilution with a concentration of 100 µg/mL by pipetting 1 mL of the standard solution into a 10 mL volumetric flask. Then dissolve it with 96% ethanol up to the mark. Pipette 0.5 mL of rutin 100 µg/mL solution, add 1.5 mL of 96% ethanol, 0.1 mL of 10% P aluminum chloride, 0.1 mL of 1 M sodium acetate and 2.8 mL of distilled water, added successively. Shake and let stand for 30 minutes at room temperature. Then measure the absorption at a maximum wavelength of 417.00 nm using UV-Vis Spectrophotometry. Perform dilutions at concentrations of 80, 100, 120, 140 and 160 µg/mL by pipetting 0.8 of the 1000 µg/mL standard solution; 1; 1.2; 1.4 and 1.6 mL. Then each solution was measured for its absorption at the maximum wavelength using UV-Vis Spectrophotometry. (Ministry of Health of the Republic of Indonesia, 2017).

**b. Total Tannin**

Catechins in the oven at 105° C until constant weight. After a constant weight of 50 mg of catechins was dissolved with ethyl acetate up to 50 mL, a standard catechin solution was obtained with a concentration of 1000 µg/mL. Then pipette 10 mL of 1000 µg/mL solution added to 100 mL of ethyl acetate to obtain a standard solution with a concentration of 100 µg/mL. To 4 mL of 100 µg/mL solution, add ethyl acetate to 10 mL (40 µg/mL). The absorption was measured at a wavelength of 279.80 nm using a UV-Vis spectrophotometer. From a solution of 100 µg/mL catechins, dilution was carried out to obtain serial solutions with concentrations of 25, 30, 35, 40 and 45 µg/mL by means of a pipette of 2.5 each; 3; 3.5; 4 and 4.5 mL of a solution with a concentration of 100 µg/mL. Put into a 10 mL flask filled up to the mark with ethyl acetate. Measure the absorption with a UV-Vis spectrophotometer at the maximum wavelength so that a calibration curve is obtained. (Lucida, 2007).

**c. Total Phenol**

Weigh approximately 10 mg of gallic acid in methanol Pa. into a 10 mL volumetric flask to obtain a concentration of 1000 µg/mL (standard solution). Perform dilution with a concentration of 50 µg/mL. Pipette 0.5 mL of a 50 µg/mL solution, add 5 mL of FolinCiocalteu 7.5% LP, let stand for 8 minutes, add 4 mL of 1% NaOH, incubate for 1 hour. Then measure the absorption at a wavelength of 732.50 nm using UV-Vis Spectrophotometry. Perform dilutions at concentrations of 20, 30, 40, 50, and 60 µg/mL by pipetting 0.2 of the 1000 µg/mL standard solution; 0.3; 0.4; 0.5, and 0.6 mL, and added methanol Pa. up to the mark in a 10 mL flask. Take 0.5 mL of each solution, then 5 mL of Folin-Ciocalteu 7.5% LP, let stand for 8 minutes, add 4 mL of 1% NaOH, incubate for 1 hour. Then measure the absorption using UV-Vis Spectrophotometry (Ministry of Health of the Republic of Indonesia, 2017).

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**RESULTS AND DISCUSSION**

A total of 8 pieces of fresh betel leaves (Fig. 1) that have been washed with running water then boiled for 15 minutes to boil over medium heat in 400 mL of water, so that a traditional betel leaf medicinal herbs is obtained. Furthermore, a phytochemical analysis of traditional medicinal ingredients from betel leaf (*Piper betleL.*) was carried out so that the following results were obtained (Fig 2.)



Fig 1. Betel Leaf



Fig 2. Process of Medicinal Herbs

Table 1. Results of Qualitative Analysis of Traditional Medicinal Herbs From Betel Leaf (*Piper betle*L.)

Chemical Compound	Reactants	Parameters	Ingredients Observation
Alkaloid	Mayer	White/yellow precipitate	(-)
	Wagner	Reddish-brown precipitate	(-)
	Bouchardat	Brown to black precipitate	(-)
	Dragendroft	Reddish -brown precipitate	(-)
Flavonoids	Test with intense red Zn powder	(flavonol 3 glycosides)	(-)
	Test with Mg powder	orange red (flavones, chalcones, aurons)	(+)
Tannins	FeCl <sub>3</sub>	Dark green or blue to black in color	(+)
Terpenoids	N-hexane, acetic anhydride, H <sub>2</sub> SO <sub>4</sub>	Red color	(-)
Steroid	N-hexane, acetic anhydride, H <sub>2</sub> SO <sub>4</sub>	Blue color	(-)
Saponin	Water, HCl 2n	Formed foam	(-)
Phenol	Folinciocalteu LP	Blackish green color	(+)
Essential Oil	KMNO <sub>4</sub>	Color turns pale or disappears	(-)
	Acetic acid hydride, H <sub>2</sub> SO <sub>4</sub>	Blue green color	(-)

Description:

(+) : Indicates the presence of a chemical compound

(-) : Indicates the absence of a chemical compound

In this study the sample betel leaves used were obtained from Surau Gadang Village, Nanggalo District, Padang City, West Sumatera and identified at the Herbarium Laboratory (ANDA) Andalas University (UNAND) in the biology department of FMIPA Andalas University. The purpose of identification is to determine the identification of the sample to be used in accordance with the Indonesian Herbal Pharmacopoeia Edition II (2017). Based on the identification results, it can be seen that the sample used in this study was betel leaf (*Piper betle*L.) belonging to the Piperaceae family.

Sampling of betel leaves is done by taking leaves that are not too young and not too old, namely leaves that are perfectly green, at which time the levels of active compounds are highest so that good quality is obtained (Rivalet *al*, 2014) then *boiled*. So that the traditional medicinal herbs of betel leaf are obtained in accordance with the traditional medicinal herb formulary (Ministry of Health of the Republic of Indonesia, 2017).

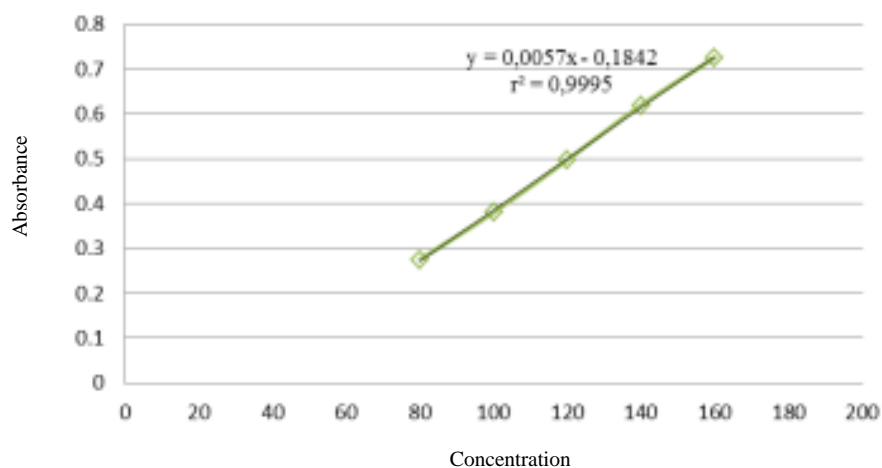
After obtaining the traditional betel leaf medicinal herbs, a qualitative analysis was then carried out to determine the chemical compounds contained in the traditional betel leaf medicinal ingredients. In testing alkaloids using the Bouchardat LP reagent, Mayer LP reagent, Wagner LP reagent, and Dragendroft LP reagent, the results were negative, because there was no color change after the medicinal ingredients were added to the reagent. In testing the flavonoids with Mg and HCl P powders, positive results were obtained indicating that the traditional medicinal ingredients of betel leaf (*Piper betle*L.) contained flavonoids of the types flavones, chalcones, and auron in which the solution turned orange-red which was previously traditional medicinal ingredients of betel leaves. orange yellow. The addition of concentrated HCl is used to hydrolyze flavonoids into their aglycones, namely by hydrolyzing O-glycosyl. The glycosyl will be replaced by H<sup>+</sup> from the acid because of its electrophilic nature. Reduction with Mg and concentrated HCl can produce red or orange complex compounds in flavonols, flavanones, flavanonols and xanthone (Robinson, 1995 & Ikalinuset *et al.*, 2015). (Table 1.)

In the tannin test with iron (III) chloride reagent, it showed a positive result with the formation of a dark green color change. The tannin test using FeCl<sub>3</sub> can show the presence of phenol groups, if there are phenolic compounds, it is also possible that tannins are present, because tannins are polyphenolic compounds (Harborne, 1987 & Ikalinuset *et al.*, 2015). The terpenoid and steroid tests showed negative results. The saponin test showed a negative result because the foam disappeared after the addition of 2N HCl and the height of the foam did not reach 1 cm. In the phenol test, a concentrated folin-ciocalteu reagent solution was used which showed a positive result with a change in color to black-green or bluish. The intensity of the blue color is determined by the amount of phenol content in the sample solution. The greater concentration of phenolic compounds in the sample, the darker the blue color appears (Singleton & Rossi, 1965 & Ismail *et al.*, 2012). In the essential oil test, negative results were obtained in all three tests, namely with KMnO<sub>4</sub> reagent, acetic acid anhydride and concentrated sulfuric acid, and in the addition of vanillin-sulfuric acid spotting solution.

## Quantitative analysis Traditional Medicine Herbs

### Total Flavonoids

In the test for determining flavonoid levels total betel leaf traditional medicinal herbs, carried out using the aluminium chloride used colorimetric method by UV-Vis spectrophotometer which was calculated as rutin. The principle of determining the levels of flavonoids by the colorimetric aluminum chloride method is the formation of a complex between aluminium chloride and the keto group on the atom C-4 and the hydroxyl group on the neighboring C-3 or C-5 atoms from the flavones and flavonol groups (Azizah *et al.*, 2014). Then the total flavonoid levels were obtained from the traditional betel leaf medicinal herbs by measuring the absorbance of the sample at a concentration of 100 µg/mL which was repeated 3 times to obtain a total flavonoid level of 35.941%. (Table 2.)



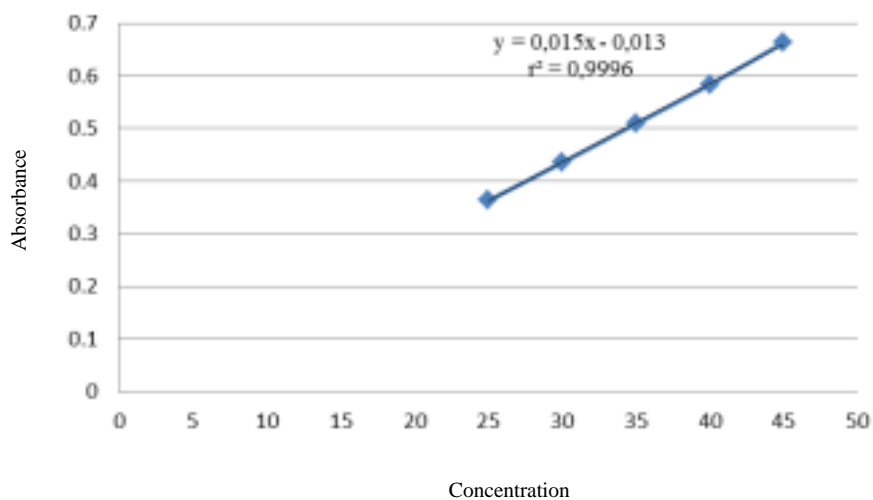
**Fig 3.** Flavonoid Calibration Curve

**Table 2.** Results of Determination of Total Flavonoid

Experiment	Concentration	Absorbance	Content Flavonoid (%)
1	100	0.016	35.122
2	100	0.027	37.052
3	100	0.019	35.649
Average	100	0.020	35.941
SD			0.997

**Total Tannin**

The next quantitative test is the determination Total tannin content was carried out using UV Vis spectrophotometry at a wavelength of 200-400 nm. Then the total tannin content was obtained from of traditional betel leaf medicinal ingredients by measuring the absorbance of the sample at a concentration of 40  $\mu\text{g/mL}$  which was repeated 3 times to obtain a total tannin content of 5.444%.

**Fig 4.** Tannin Calibration Curve**Table 3.** Results of Determination of Total Tannin

Experiment	Concentration	Absorbance	Content (%)
1	40	0.029	7.000
2	40	0.016	4.833
3	40	0.014	4.500
Average	40	0.019	5.444
SD			1.357

### Total Phenolic

The next quantitative test is the determination total phenolic content was carried out using the folin-ciocalteu reagent based on the reducing power of the phenol hydroxyl groups using standard gallic acid. Then the total phenol content was obtained from the traditional betel leaf medicinal herb by measuring the absorbance of the sample at a concentration of 50 µg/mL which was repeated 3 times to obtain a total phenol content of 2.905%.

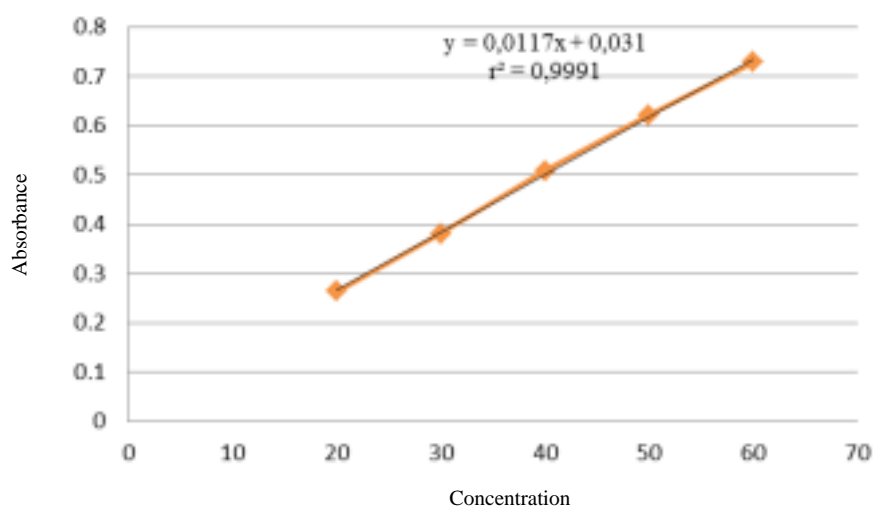


Figure Phenolic calibration curve

Table 3. Results of Determination of Total Tannin

Experiment	Concentration	Absorbance	Content (%)
1	50	0.049	3.076
2	50	0.046	2.564
3	50	0.049	3.076
Average	50	0.048	2.905
SD			0.295

According to Cowan (1999) secondary metabolites from plants that have potential as antibacterials are phenolics, polyphenols, quinones, flavones, flavonoids, tannins, coumarins, terpenoids, alkaloids, lectins and polypeptides. Used flavonoid include protecting cell structures, increasing the effectiveness of vitamin C, anti-inflammation, preventing bone loss and as antibiotics (Pourmourad (2006) & Haris (2011)). According to Kurniasari's research (2006) stated that a number of medicinal plants containing flavonoids have been reported to have antioxidant, antibacterial, antiviral, anti-inflammatory, antiallergic, and anticancer activities.

Flavonoids work as antibacterials with several mechanisms of action, including inhibiting nucleic acid synthesis, inhibiting the function of the cytoplasmic membrane and inhibiting the energy metabolism of bacteria, namely forming complexes with extracellular proteins from the bacterial cell wall (Maniket *et al.*, 2014 & Jayalakshmi *et al.*, 2015)

Based on the results of the research that has been done, it was found that traditional medicinal herbs from betel leaf (*Piper betle*L.) contain 35.941% flavonoids, 5.444% tannins, and 2.905% phenols. The content of flavonoids in the sample is quite high so that the traditional medicinal herbs from betel leaf (*Piper betle*L.) can be declared as a traditional medicine to treat acne. Because as it is known that the factors that cause the development of acne one of them by increasing sebum production and lipolysis of bacteria from sebum triglycerides. The accumulation of sebum in the sebaceous follicles facilitates the proliferation of anaerobic bacteria, one of which is *Propionibacterium acnes*, causing inflammation (Dipiroet *et al.*, 2015).

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## CONCLUSION

The chemical compounds contained in the traditional betel leaf medicinal herbs showed positive results in the testing of flavonoids, tannins and phenolic compounds. Flavonoid compounds are known to have antibacterial activity so they are thought to play a role in treating acne.

The results of the quantitative analysis of the traditional medicinal herbs of betel leaf obtained a total flavonoid compound content of  $35.941\% \pm 0.997$ , a total tannin content of  $5.444\% \pm 1.357$ , and a total phenolic content of  $2.905\% \pm 0.295$ .

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